Assessment of Isoform specificity for a polyclonal Elastase ELISA

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Introduction: Elastase is secreted from the pancreas and passes through the GI-tract without significant degradation. Exocrine pancreatic function analysis can therefore be performed with Elastase stool tests. Five different isoforms of human pancreatic elastase (PA I, IIA, IIB, IIIA, IIIB) have been identified. Three different polyclonal antisera that are used in a commercial ELISA were investigated for their specific recognition of human elastase isoforms in human pancreatic juice.

Material and Methods: Secreted proteins from human pancreatic juice were analysed by one or two-dimensional gel-electrophoresis, followed by westernblot analysis using 3 different polyclonal anti-Elastase antibodies (BIOSERV GmbH, Germany) and MALDI-TOF-MS. Elastase-activity was analysed in immunoprecipitates from human pancreatic juice using a fluorogenic Elastase substrate. Finally, cross-reactivity of the antibodies was tested against pancreatin from pig pancreas.

Results: In 1D Western blots of pancreatic juice all three polyclonal antisera against human Elastase detected a single ~30kDa protein. Immunoprecipitates with these antibodies exhibited elastase activity as determined with the fluorogenic Elastase substrate. In 2D-Westernblots (pH3-10) proteins in the molecular weight range of ~30 kDa were separated into a number of spots of different isoelectric points (pI). MALDI-TOF-MS-Analysis of these spots revealed the presence of pancreatic Elastase IIIA and IIIB isoforms, but not Elastase II or Elastase I isoforms. Western blot analysis of pancreatin from pig pancreas revealed no cross-reactivity with any of the three antisera tested.

Conclusion: All three commercial antibodies that are used in a polyclonal Elastase ELISA preferentially detect human Elastase isoforms IIIA and IIIB, and do not cross-react with pig pancreatin. At present differences concerning expression and specific function of PA II or PA III isoforms are still unknown, but we could demonstrate, that PA III isoforms clearly possess Elastase activity as determined by a fluorogenic Elastase substrate. Elastase I ia not an enzyme expressed in the adult human pancreas and should therefore not be referred to in commercial test kits for exocrine pancreatic function.

Detection of pancreatic Elastases 3A and 3B by a polyclonal Elastase ELISA

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Subject

Exocrine pancreatic insufficiency is a frequent consequence of chronic or severe acute pancreatitis. Assessment of exocrine pancreatic function is commonly performed using Elastase stool tests which have largely replaced invasive function testing. Pancreatic Elastase is a secreted enzyme of the exocrine pancreas which passes through the intestine without significant degradation. Measurement of fecal elastase therefore reliably reflects clinically relevant degrees of pancreatic insufficiency.

To date the most widely used assay for elastase measurement has been a commercially available "Pancreatic Elastase 1" ELISA. A competing commercial Elastase ELISA using polyclonal antisera has been reported to be of higher specificity (1). However, in a recent publication it was speculated (2) that this assay recognises an unknown antigen epitope that is different from pancreatic elastase 1.

Several experimental facts complicate matters further: The human pancreatic elastase 1 gene is transcriptionally silent and to date elastase 1 expression has only been detected in skin keratinocytes but never in the adult human pancreas.

Recombinant Elastase 2B was recently shown to be devoid of proteolytic activity (3)

Considerable controversy exists to date concerning the identity of the main pancreatic elastase activity and the identity of elastase isoforms which are recognised by elastase stool ELISAs.

We therefore tested the polyclonal antisera used in the polyclonal Elastase ELISA for their specific recognition of human elastase isoforms in human pancreatic juice.

Literature

1 J-U Hahn et al. (2006) Pancreas 30, 189.
2 A. Schneider et al. (2005) Clinical Chemistry 51, 1.
3 E. Szepessy et al. (2006) Pancreatology 6, 117.

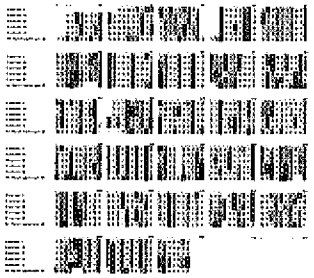
Elastases form a subfamily of serine proteases that hydrolyze many proteins in addition to elastin. The human genome contains six elastase genes which encode the structurally similar proteins elastase 1 (keratinocytes); 2 (neutrophiles); 2A, 2B, 3A, and 3B (all pancreatic). Unlike other elastases, elastase 3B has little elastine-cleaving (elastolytic) activity, but is secreted as a precursor zymogen and may perform other digestive functions in the gut. Both elastase 3A and elastase 3B have, erroneously, been referred to as protease E and as elastase 1. The human pancreatic elastase 1 gene is transcriptionally silenced and to date elastase 1 expression has only been detected in skin keratinocytes but never in the adult pancreas. Clinical literature that reports on human elastase 1 activity in the pancreas therefore is actually referring to other elastase isoforms (2A, 2B, 3A, and 3B).

Sequence homology table

	2A	2B	3A	3B	CT
1	54	51	51	51	32
2A		89	56	56	36
2B			54	53	33
3A				92	39
3B					39

Indicated are the percentage identities of protein sequences of human Elastases 1,2A,2B,3A, 3B and human Chymotrypsin

Sequence-Alignment of Elastase Isoforms and Chymotrypsin

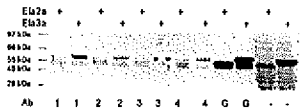


Results

Polyclonal Elastase Ab detect a ~30 kDa protein in human pancreatic juice

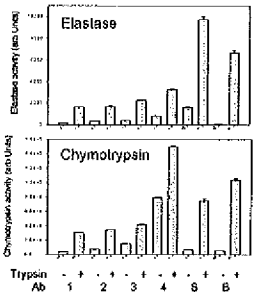
Human pancreatic juice was separated by SDS-PAGE, blotted onto nitrocellulose membrane and detected with Elastase antisera against Peptide 1,2,3,4 (rabbit; 2µg/ml).

Immune precipitation of recombinant Elastase isoforms 2A and 3A by polyclonal Elastase Ab



HEK-293 cells, transiently transfected with expression constructs pReceiver-Ela2a-GFP or pReceiver-Ela3a-GFP were lysed and immune precipitations with Elastase antisera 1,2,3,4 (rabbit; 2µg) and monoclonal anti-GFP Ab (mouse; 0.5µg) were performed. Following SDS-Page, Western-blotting was done with anti-GFP Ab.

Trypsin activation of Elastase and Chymotrypsin activity in anti-Elastase immune precipitations

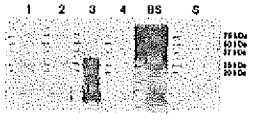


Elastase was immunoprecipitated from equal volumes of human pancreatic juice with the following antibodies (Elastase polyclonal antisera against Peptide 1,2,3,4 (rabbit; 2µg/ml), monoclonal -Ab S (mouse), polyclonal -Ab B (rabbit). Elastase activity was measured with the fluorogenic substrate R110-Ala$_4$.Chymotrypsin activity was measured with the fluorogenic substrate AMC-(Suc-Ala2-Pro-Phe)

Homology human/pig Elastases

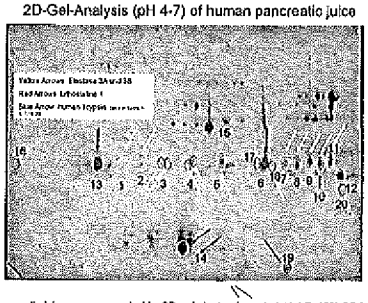
Elastase 1(pig)	68%	Elastase 1
	56%	Elastase 2A
	62%	Elastase 2B
	51%	Elastase 3A
	50%	Elastase 3B
Elastase 2 (pig)	82%	Elastase 2A
	76%	Elastase 2B
	58%	Elastase 3A
	54%	Elastase 3B
	53%	Elastase 1

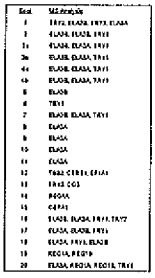
Cross reactivity of polyclonal Elastase Ab with oral enzyme replacement (Panzytrat)



250 µg Panzytrat / Lane were separated by SDS-PAGE and blotted onto nitrocellulose membrane and detected with different elastase antisera against Peptide 1,2,3,4 (rabbit; 2µg/ml),polyclonal -Ab (rabbit; 1:500), monoclonal –Ab S (mouse; 1:500)

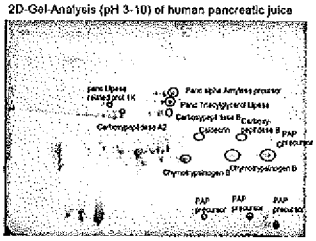
2D-Gel-Analysis (pH 4-7) of human pancreatic juice



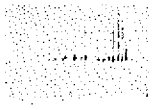


30 µl of pancreatic juice were separated by 2D-gel electrophoresis (pH 4-7, 12% SDS-Page) and blotted onto PVDF membrane. After ink-staining MS-Analysis of selected protein spots was performed using LC-ESI-MS (QStar und FT-ICR) and results were analysed with the "Sequest" program against SwissProt Database.

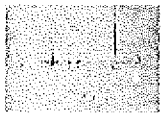
2D-Gel-Analysis (pH 3-10) of human pancreatic juice



30 µl of pancreatic juice were separated by 2D-gel electrophoresis (pH 3-10, 12% SDS-Page) and blotted onto PVDF membrane. After ink staining MS-Analysis of selected protein spots was performed using LC-ESI-MS (QStar und FT-ICR) and results were analysed with the Sequest program against SwissProt Database.



(representative) Reblot with anti-Elastase Ab; Peptide 1 (rabbit; 2µg/ml).



Reblot with anti-Trypsin Ab; Chemicon (rabbit; 2µg/ml).

Conclusion

Four different polyclonal antisera used in a commercial Elastase ELISA recognise ~ 30 kDa proteins with endogenous enzymatic activities against the specific fluorogenic Elastase substrate R110-Ala$_4$. Mass-spectrometric analysis identified these proteins as human Elastase isoforms 3A and 3B.

Due to a slight cross reactivity against porcine pancreatin one of the four antisera was removed from the test system. Cross reactivity against Chymotrypsin is comparable to a commercial monoclonal antibody or a different polyclonal anti-Elastase antiserum

At present, differences concerning expression, secretion and specific function of pancreatic elastase 2 and 3 isoforms in humans are still unknown. and their specific prognostic value in the assessment of exocrine pancreatic insufficiency needs further evaluation.